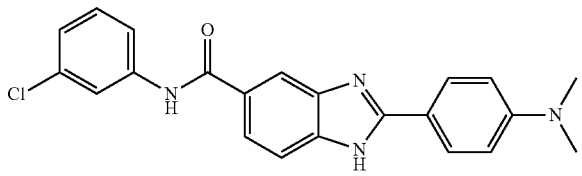
Example 46

[0381] 2-(4-dimethylamino-phenyl)-1H-benzoimidazole-5-carboxylic acid (3-chloro-phenyl)-amide



2-(4-dimethylamino-phenyl)-1H-benzoimidazole-5-carboxylic acid (3-chloro-phenyl)-amide was synthesized from 2-(4-dimethylamino-phenyl)-1H-benzoimidazole-5-carboxylic acid and 3-chloro-phenylamine by following the procedure for example 1. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.90 (s, 1H), 10.37 (s, 1H), 8.17 (s, 1H), 8.08-7.98 (m, 3H), 7.82-7.73 (m, 2H), 7.61 (d, J=8.3 Hz, 1H), 7.39 (t, J=8.1 Hz, 1H), 7.15 (ddd, J=8.0, 2.1, 0.9 Hz, 1H), 6.91-6.81 (m, 2H), 3.02 (s, 6H). MS m/z (M+H) 390.9.

2/Biological Activity

2-1/In Vitro Determination of Compound Activity by HRTF Assay

[0382] Principle:

In the experiment, recombinant Erk1 and Erk2, tagged with a polyhistidine stretch in their N-terminal part were used. To reproduce the interaction between ERK1/2 and Myd88, a peptide corresponding to amino acids 24-40 of Myd88 was used: biotin-Ahx-PLAALNMRVRRRLSLFLNVR with Ahx being aminohexanoic acid as a spacer (biotin-MyD88peptide). This peptide encompassed the interaction motif of Myd88 with the CD domain of ERK1/2. To perform the HTRF assay this peptide was N-terminally labelled with biotin. To detect the interaction between the peptide and Erk1 or Erk2 the following components were used: an antibody directed against the His-tag, labelled with the donor fluorophore (Europium cryptate); The streptavidine protein, labelled with the acceptor XL665 fluorophore directed against the biotin. All the components except Erk1, Erk2 and the peptide were purchased from Cisbio (Condolet, France).

[0383] Homogeneous Time Resolved Fluorescence (HTRF) assay combines two techniques: Fluorescence Resonance Energy Transfer (FRET) and Time Resolved (TR) measurement. In TR-FRET, a transfer of fluorescence between a donor and an acceptor molecule generates a signal that can then be measured. This energy transfer is only possible when both fluorophores are close enough to each other. Therefore, this method can be used to detect protein-protein interaction.

[0384] Experimentation:

For MyD88-ERK1 interaction the reaction medium contained: 6xHis-ERK1 at 7.5 nM, biotin-MyD88peptide at 25 nM, anti-Histidine-Cryptate antibody at 1.3325 nM and XL665 labeled-Streptavidine at 3.125 nM.

For MyD88-ERK2 interaction the reaction medium contained: GST-ERK2 at 50 nM, biotin-MyD88pep at 50 nM, anti-GST-Cryptate antibody at 0.8 nM and XL665 labeled—Streptavidine at 12.5 nM.

[0385] Concentrations of compounds to be tested ranged from 0.1 μM to 100 μM. Stock solutions of compounds according to the invention were prepared at 10 mM in DMSO. Compounds stock solution were directly distributed in black 384-well plate (Greiner low-volume) in Tris-HCl 20 mM, pH 8/0.1% BSA/0.05% Tween 20/150 mM NaCl (ERK1 assay) or in Tris-HCl 10 mM, pH8/0.1% BSA/0.05% Tween 20/150 mM NaCl (ERK2 assay) by an HP D300 Digital Dispenser to obtain the required final concentrations. Proteins (ERK1, ERK2) were then added and the plate was incubated for 30 min at room temperature. After incubation, biotin-Myd88peptide, anti-his-K, anti-GST-k and XL665-Streptavidin were added to each corresponding well to reach a final volume of 20 μL. The plate was incubated at room temperature for 2 hours and fluorescence was measured using a TECAN Infinite F500 Reader®.

[0386] Results are calculated as the variation of fluorescence (Delta F) between control and samples. Delta F was calculated using the following equation: [(Ratio sample-Ratio of the arithmetic mean of the control)/Ratio of the arithmetic mean of the control]×100 where ratio=(Acceptor fluorescence/Donor fluorescence)×10000. The IC50 were determinated using GraphPad software following this equation: log (inhibitor) versus response variable slope (jour parameters) y=Bottom+[(Top-Bottom)/(1+10((log IC50−X) hillslope)]. Bottom was determinated by the equation and Top was the DMSO control value. The results are presented below at paragraph 2-5.

2-2/Cell-Based Assay

[0387] The terms "SRE promoter" refer to "Serum response elements", which is a DNA sequence (CC(A/T) 6GG) that is used upstream of reporter genes and is known to be the binding site of the SRF (Serum response factor) proteins encoded by SRF gene identified by Gene Bank number (Gene ID: 6722, NM_003131.2). To determine the activity of the compounds on ERK1/2, Fluman Colon Tumor (HCT116) p53+/+ cell line stably transformed to express the luciferase reporter gene under the control of the serum response element (SRE) was used. This cell line was cultured in McCoy's medium supplemented with 10% FBS, 2 mM glutamine, 1% antibiotics and 1 μM of puromycin.

[0388] HCT116 p53+/+ SRE luciferase cells were plated in 96-well flat-bottom plate at 20,000/well and incubated at 37° C. for 36 hours. The medium was discarded and replaced by 100 μl of serum free medium for 16 hours. Cells were then pretreated for 2 hours with the compounds according to the invention at various concentrations (from 0.001 μM to 80 μM). Cells were then stimulated by addition of 10% FCS and incubated at 37° C. for four additional hours and the expression of reporter gene was assessed using the One-Glo Luciferase reagent (Promega). Luminescence was quantified after 5 minutes in a TECAN infinite M200 Reader (Mannedorf, Switzerland). The results are presented below at paragraph 2-5.

2-3/Apoptosis Assay

[0389] Cellular apoptosis was measured using the Caspase-Glo® 3/7 assay kit (Promega). To perform this study, HCT116 p53+/+ cells were seeded at a density of 7,000 cell/well in 96-half wells plate and maintained 24 h at 37° C. in McCoy's medium supplemented with 2 mM glutamine, 1% penicillin, 1% of streptomycin and 10% FBS.